Identification of human platelet α_2 -adrenoceptors with a new antagonist [3 H]-RX821002, a 2-methoxy derivative of idazoxan

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1 The binding of a new α_2 -adrenoceptor antagonist, [3 H]-RX821002 (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline), was investigated in human platelet membranes and compared with [3 H]-yohimbine binding parameters.

2 Analysis of kinetic data revealed association and dissociation time courses consistent with a simple bimolecular reaction. Saturation isotherms showed that [3 H]-RX821002 labelled a higher total number of α_2 -binding sites (224 ± 31 vs 168 ± 24 fmol mg⁻¹ protein) than [3 H]-yohimbine and with higher affinity (K_d : 0.92 ± 0.06 vs 1.51 ± 0.08 nM). Moreover [3 H]-RX821002 exhibited a lower percentage of non-specific binding.

3 The difference in total binding is due to a better labelling of the α_2 -adrenoceptors in the low affinity state by [3 H]-RX821002 since the labelled receptors number in high affinity state was identical with the two radioligands.

4 [3 H]-RX821002 binding displayed a specificity similar to that obtained with [3 H]-yohimbine. The potency of various compounds acting on adrenoceptors was: yohimbine > oxymetazoline > UK14304 > (-)-adrenaline > prazosin $\geq$ (+)-adrenaline > isoprenaline. This order of potency is classical for an α_{2A} -adrenoceptor.

5 RX821002 is a more potent α_2 -adrenoceptor antagonist than yohimbine on adrenaline-induced platelet aggregation.

6 These results indicate that [3 H]-RX821002 is a suitable ligand for the identification of human platelet α_2 -adrenoceptors.

Introduction

It is now well established that human platelets possesses both β - and α_2 -adrenoceptors which mediate opposite effects on adenosine 3':5'-cyclic monophosphate (cyclic AMP) production via activation or inhibition of the enzyme adenylate cyclase (Jakobs *et al.*, 1978; Steer & Atlas, 1982; Lenox *et al.*, 1985; Lanza & Cazenave, 1987). Platelet α_2 -adrenoceptors have been characterized with various α_2 -antagonist radioligands ([3 H]-dihydroergocryptine, [3 H]-yohimbine, [3 H]-rauwolscine, [3 H]-phenolamine and [3 H]-idazoxan) (Alexander *et al.*, 1978; Steer *et al.*, 1979; Lanza & Cazenave, 1987; Michel *et al.*, 1989). Although these drugs display high affinity for the α_2 -adrenoceptor, some discrepancies have been reported concerning the total binding capacities defined with the ligands. For example [3 H]-dihydroergocryptine was found to label 2 or 3 fold more sites than [3 H]-yohimbine (Motulsky & Insel, 1982; Boon *et al.*, 1983). Moreover, some of these compounds also interact with other binding sites in addition to α_2 -adrenoceptors (Couprie *et al.*, 1987; Convents *et al.*, 1989; Langin & Lafontan, 1989; Michel *et al.*, 1989).

Platelet α_2 -adrenoceptors have been used as an adrenergic index for α_2 -adrenoceptors located on various tissues although this technique has been the subject of some controversy (Hamilton & Reid, 1986; Nutt, 1987; Crampes *et al.*, 1989). Moreover, comparative studies using [3 H]-yohimbine have shown that this compound is not adequate since it is not suitable for α_2 -adrenoceptor identification in animal platelets (Kerry *et al.*, 1984) or in some other tissues such as nervous or adipose tissues (Cheung *et al.*, 1982; Bylund, 1985; Langin & Lafontan, 1989). Recently, our group demonstrated that the new α_2 -adrenoceptor antagonist, RX821002 (a 2-methoxy derivative of idazoxan; Stillings *et al.*, 1985) was able to identify rat and hamster fat cell α_2 -adrenoceptors on which [3 H]-

yohimbine binding was unsuccessful (Saulnier Blache *et al.*, 1989).

We describe here experiments concerning the efficacy of RX821002 in inhibiting adrenaline-induced platelet aggregation. We also compared the binding characteristics of [3 H]-RX821002 and [3 H]-yohimbine to human platelet α_2 -adrenoceptors and demonstrated that this new radioligand displayed all the characteristics for labelling α_{2A} -adrenoceptors.

Methods

Platelet membrane preparation and binding procedure

Blood was taken by antecubital venopuncture from six healthy male volunteers aged from 25 to 35 years. The platelet membranes were prepared as follows: 20–30 ml of blood was collected over 2 ml of 0.16 M sodium citrate solution and immediately centrifuged at 160g for 10 min at room temperature. The platelet rich plasma (PRP) was collected and the platelet membranes were prepared as previously described (Villeneuve *et al.*, 1985).

The platelet membranes were used for adrenoceptor determination by a radioligand binding technique as previously described by Berlan & Lafontan (1985). Total binding of [3 H]-yohimbine and of the new α_2 -adrenoceptor antagonist [3 H]-RX821002 was determined by incubating 100 μ l aliquots of the resuspended membrane preparation (100–200 μ g protein assayed by the method of Lowry *et al.*, 1951) with the radioligand in a total volume of 400 μ l of binding buffer (50 mM Tris-HCl, 0.5 mM MgCl₂, pH 7.5). Specific binding was defined as the difference between total and non-specific binding determined in parallel with an excess (10 μ M) of phenolamine. Incubations were carried out for 20 min at 25°C under constant shaking at 100 cycles per min. The samples were filtered through fibreglass filters (Whatman GF/C) on a Millipore manifold sampling unit. The filters were washed twice with

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10 ml of cold binding buffer and the radioactivity trapped on the filter was counted in a Beckman spectrometer with an efficiency of 35%. The number of sites ($B_{\max}$) and K_d values were calculated with a computer-assisted analysis of binding at saturation and according to Scatchard plots. In competition experiments, various compounds acting at adrenoceptors were dissolved in the binding buffer, diluted and added to the assay (100 μ l) just prior to the experiments.

Platelet aggregation

Platelet aggregation studies were described previously (Villeneuve *et al.*, 1985). Briefly, 10 ml of venous blood was collected over 2 ml of 0.16 M sodium citrate solution and immediately centrifuged at 160 g for 10 min at room temperature. The platelet number was adjusted to 350,000–400,000 mm^{-3} with autologous platelet-poor plasma [obtained after centrifugation (10,000 g for 5 min) of the residual blood collected after the first centrifugation] and platelet aggregatory responses to adrenaline (6 μ M) were examined by a turbidimetric method using a Coultronic apparatus. After this, the effects of increasing concentrations of yohimbine or RX821002 (0.006 to 6 μ M) were studied on the same fresh platelet preparation. Drugs were added to the PRP 5 min before the addition of adrenaline. The aggregatory response was followed until the complete aggregation of the platelets. The velocity of platelet aggregation was measured as the variation of the optical transmission recorded on the chart of the aggregatometer (mm per unit time).

Drugs and chemicals

[^3H]-yohimbine (90 Ci mmol^{-1}) was obtained from Amersham (Amersham, England) and [^3H]-RX821002 (2-(2-methoxy-1,4 benzodioxan-2-yl)-2-imidazoline) (43.8 Ci mmol^{-1}) was a gift from Reckitt and Colman Labs (Kingston-upon-Hull, U.K.). (+)- or (–)-adrenaline bitartrate came from Winthrop (Rensselaer, U.S.A.); phentolamine mesylate was obtained from Ciba Geigy (Basel, Switzerland), UK14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate) and prazosin from Pfizer (Sandwich, U.K.), (–)-isoprenaline and yohimbine hydrochloride from Sigma (France), oxymetazoline from Schering (Kenilworth, NJ, U.S.A.).

Data analysis

Values are means $\pm$ s.e.mean. Statistical analysis was by paired t test and differences were considered significant when P was less than 0.05.

Results

Effect of yohimbine and RX821002 on adrenaline-induced aggregation in human platelets

The aggregatory velocity of adrenaline was dose-dependently reduced by increasing concentrations of yohimbine and RX821002 (Figure 1) indicating a progressive occupation of platelet α_2 -adrenoceptors. However, the aggregatory sensitivity to adrenaline was more affected by increasing concentrations of RX821002. For a given concentration (between 0.006 and 0.6 μ M) of each compound, the reduction of velocity of the aggregatory response to adrenaline was 2.28 ± 0.38 times greater with RX821002 than yohimbine ($n = 3$). With the higher dose of RX821002 (6 μ M), the aggregatory response to an equimolar dose of adrenaline completely disappeared whereas the aggregatory response persisted with yohimbine.

Comparative parameters of [^3H]-yohimbine and [^3H]-RX821002 binding

Kinetic studies of [^3H]-RX821002 binding Three independent studies of the association and dissociation time course were performed. Data from both association and dissociation

Figure 1 Effect of various concentrations (0.006 to 6 μ M) of yohimbine (A) or RX821002 (B) on the aggregatory response induced by 6 μ M adrenaline in human platelets. Antagonists were added to the platelet rich plasma (PRP, 400,000 platelets mm^{-3}) 5 min before the addition of adrenaline (indicated by the arrows).

processes were best fitted by a mono-exponential model. The binding of [^3H]-RX821002 to human platelet membrane preparations was very rapid and reached a steady-state within 10 min (Figure 2a). The mean value of the rate constant of the pseudo-first order association reaction (K_{on}) was $0.123 \pm 0.013 \text{ min}^{-1}$. Dissociation of [^3H]-RX821002 binding was rapidly reversed when 10 μ M phentolamine was added after equilibrium was reached (Figure 2b) and the mean value of k^{-1} was $0.287 \pm 0.06 \text{ min}^{-1}$. The calculated kinetic constant of the association reaction ($k + 1$) was $0.093 \pm 0.015 \text{ min}^{-1} \text{ nM}^{-1}$ and the kinetically derived equilibrium dissociation constant (K_d) was $2.80 \pm 0.32 \text{ nM}$. This value is in good agreement with the K_d value given by the analysis of saturation binding data.

Comparative saturation binding studies of [^3H]-RX821002 or [^3H]-yohimbine The equilibrium dissociation constants (K_d) and total number of binding sites ($B_{\max}$ values) for the [^3H]-yohimbine and [^3H]-RX821002 were measured on the same batch of platelet membranes. The results (obtained in 6 separate experiments) are summarized in Table 1. Binding conditions were similar to those described under Methods using 50 mM Tris-HCl, 0.5 mM MgCl_2 as binding buffer and 200 μ M adrenaline was used instead of phentolamine for definition of non-specific binding.

The two tritiated compounds gave saturable specific binding of high affinity (Figure 3). At the K_d value, the percentage of non-specific binding was lower for [^3H]-RX821002 ($2.7 \pm 0.3\%$) than for [^3H]-yohimbine ($10.4 \pm 0.8\%$). The K_d

Figure 2 Kinetics of [^3H]-RX821002 binding to human platelet membranes. (a) Association time-course of specific [^3H]-RX821002 binding. Cell membrane preparation was incubated at 25°C with 3–4 nM of radioligand. Specific binding was determined in triplicate at the indicated time. (b) Dissociation time-course of specific [^3H]-RX821002 binding. After reaching equilibrium, 10 μM phentolamine was added to the assay and specific binding was determined in triplicate at the indicated time. Data were fitted by the microcomputer programmes HYPMIC (for association) and EXPOFIT (for dissociation) (Barlow, 1983).

values obtained with [^3H]-RX821002 in saturation experiments or in kinetics studies were practically the same. Typical saturation isotherms for each radioligand are shown in Figure 3. Scatchard plots yielded straight lines and the Hill coefficients ($n\text{H}$) were not different from unity. [^3H]-RX821002 exhibited a K_d (0.92 ± 0.06 nM) which was slightly lower ($P < 0.001$) than that of [^3H]-yohimbine (1.51 ± 0.08 nM). The maximum number of [^3H]-RX821002 binding sites (B_{max}) was $33 \pm 4\%$ ($P < 0.01$) higher than that defined with [^3H]-yohimbine.

Specificity of [^3H]-RX821002 binding to human platelet membranes

[^3H]-RX821002 specificity was studied by testing the ability of various compounds acting at adrenoceptors to impair its binding on platelet membranes (Table 2). The rank order of potency of the adrenoceptor agonists (UK14304 > (–)-adrenaline > (+)-adrenaline $\gg$ isoprenaline) demonstrated the α_2 -adrenoceptor specificity of this compound. Moreover, the stereospecificity expected for adrenaline was also observed.

Figure 3 Comparison of the specific [^3H]-RX821002 (■) and [^3H]-yohimbine (□) binding to human platelet membranes. Saturation experiments were performed as described in Methods. Insert shows Scatchard plots of specific binding derived from the saturation curves. Results are the mean of 6 separate experiments carried out in duplicate on the same platelet membrane preparation.

Finally, we tested the α_2 -adrenoceptor property of [^3H]-RX821002 binding (on this model of α_2 -adrenoceptor which is now accepted as a prototype of an α_{2A} -adrenoceptor) using yohimbine, oxymetazoline and prazosin which are the reference compounds defined by Bylund (1985) and Bylund *et al.* (1988) for α_2 -adrenoceptor subtype characterization.

The competition curves are shown in Figure 4 and the K_i values obtained after computerized analyses of the curves were 1.2 ± 0.1 vs 0.64 ± 0.16 nM for yohimbine, 4.8 ± 0.4 vs

Table 2 Inhibition of [^3H]-RX821002 binding on human platelet membrane by various compounds acting at adrenoceptors

Compounds	K_i (nM)	$n\text{Hill}$
Yohimbine	1.2 ± 0.1	1.05 ± 0.02
Oxymetazoline	4.8 ± 0.4	0.97 ± 0.04
UK 14.304	33 ± 7	0.64 ± 0.04
(–)-Adrenaline	79 ± 9	0.60 ± 0.02
Prazosin	1102 ± 100	1.05 ± 0.20
(+)-Adrenaline	1768 ± 430	0.56 ± 0.01
Isoprenaline	28500 ± 2050	0.61 ± 0.02

Inhibition studies were performed in the presence of 2–3 nM [^3H]-RX821002. The antagonist inhibition data were analysed using INHIBITION, a computerized curve fitting programme to a one-site inhibition model (Barlow, 1983). Inhibition constants were calculated from the equation: $K_i = \text{EC}_{50}/(1 + [\text{^3H-RX821002}]/K_D)$, where EC_{50} is the concentration of drug displacing 50% of bound radioligand and K_D the affinity of [^3H]-RX821002 determined in saturation experiments. The values reported are means $\pm$ s.e.mean of 3 determinations.

Table 1 Comparison of the total binding of [^3H]-RX821002 and [^3H]-yohimbine to human platelet membranes

	K_d (nM)	B_{max} (fmol mg^{-1} prot)	$n\text{Hill}$	Non-specific (%)
[^3H]-RX821002	$0.92 \pm 0.06^{**}$	$224 \pm 31^*$	1.01 ± 0.01	$2.7 \pm 0.3^{***}$
[^3H]-yohimbine	1.51 ± 0.08	168 ± 24	1.01 ± 0.01	10.4 ± 0.8

* P significantly different from yohimbine values.

*** $P < 0.005$, ** $P < 0.01$, * $P < 0.02$

Non-specific = percentage of non-specific binding at the K_d concentration.

Platelet membranes were incubated for 30 min at 25°C with increasing concentrations of radioligands as described in Figure 4. The total number of binding sites was calculated from Scatchard plots. Results were obtained from 6 different membrane preparations. Values are the mean $\pm$ s.e.mean.

Figure 4 Inhibition of [3 H]-yohimbine (a) and [3 H]-RX821002 (b) binding to human platelet membranes by yohimbine ($\square$), oxymetazoline ($\blacklozenge$) and prazosin ($\blacksquare$). The data are expressed as a percentage of the specific binding in the absence of competitor. The inhibition curves are fitted by a one-site model (Barlow, 1983). Values are means of 3 separate determinations; s.e. mean shown by vertical bars.

3.0 ± 0.9 nM for oxymetazoline and 1102 ± 100 vs 2290 ± 500 nM for prazosin on [3 H]-RX821002 and [3 H]-yohimbine binding respectively. Yohimbine and oxymetazoline exhibited a selectivity which was approximately 250–750 fold higher than that obtained with the classical α_1 -adrenoceptor antagonist, prazosin.

Identification of low and high affinity state of human platelet α_2 -adrenoceptor

In order to understand the reasons for the lower [3 H]-yohimbine $B_{\max}$ values, a series of competition studies on [3 H]-yohimbine and [3 H]-RX821002 binding were performed with the full α_2 -adrenoceptor agonist, UK14304. Inhibition of the binding of both tritiated antagonists was investigated with 20–22 different concentrations of agonist competitor and the results were analysed by a computer-assisted curve fitting

Table 3 Definition of high and low affinity sites of the human platelet α_2 -adrenoceptor evaluated by competition of [3 H]-RX821002 and [3 H]-yohimbine binding by the agonist UK14304

	K_{IH} (nM)	K_{IL} (nM)	%RH	n_{Hill}
[3 H]-RX821002	0.39 ± 0.19	77 ± 25	21 ± 3	0.63 ± 0.04
[3 H]-yohimbine	5.3 ± 2.4	132 ± 39	34 ± 4	0.53 ± 0.03

Inhibition studies of [3 H]-RX821002 (3 nM) and [3 H]-yohimbine (5 nM) binding were performed on 7 different membrane preparations with 20–22 concentrations of UK14304 (10^{-11} to 10^{-4} M). The displacement binding curves were fitted using the TWOSITEINHIB computerized programme (Barlow, 1983). K_{IH} and K_{IL} which represent the inhibition constants at the high and low affinity state receptors respectively, were calculated from the equation: $K_i = EC_{50}/(1 + [^3H \text{ antagonist}]/K_D)$, where EC_{50} is the concentration of drug displacing 50% of bound radioligand at each site and K_D the affinity of each 3H antagonist determined in saturation experiments. The proportion of the receptors in the high affinity state (RH) are given as a percentage. Values are mean $\pm$ s.e. mean.

method for one- and two-site models. The results are summarized in Table 3. As predicted by the Hill coefficients, analysis of the data indicated that displacement curves of [3 H]-RX821002 and [3 H]-yohimbine by UK14304 ($n_H = 0.630 \pm 0.04$ and 0.530 ± 0.03 respectively) were best fitted by a two-site model. The use of the TWOSITEHIB computer programme (Barlow, 1983) revealed that the percentage of high affinity sites (%RH) was slightly lower with [3 H]-RX821002 than with [3 H]-yohimbine (21 ± 3 vs $34 \pm 4\%$, $P < 0.05$, $n = 7$). However, the number of receptors in the high affinity state was nearly the same with both radioligands (calculated with the percentage of RH and the $B_{\max}$ of each radioligand).

Discussion

The aim of the study was to define the α_2 -blocking potency of a new α_2 -adrenoceptor antagonist, RX821002, on the human platelet as well as its affinity for platelet α_{2A} -adrenoceptors.

The idazoxan derivative RX821002 exhibited higher affinity for human platelet α_2 -adrenoceptors in biological assays as well as in binding experiments (Table 1) than the classical antagonist yohimbine. The pharmacological characteristics of this new α_2 -antagonist were evaluated in binding studies with its tritiated form.

Kinetic experiments indicate that binding was rapid and reversible. The time course of binding is well described as a single exponential process, suggesting that radioligand-receptor interactions are consistent with a simple bimolecular process.

Saturation isotherms indicate that [3 H]-RX821002 labels a single population of binding sites with high affinity ($K_d = 0.92 \pm 0.06$ nM); the total number of sites is higher than that defined with [3 H]-yohimbine ([3 H]-RX821002 binding being 33% higher). In order to explain the labelling of the higher number of α_2 -sites by [3 H]-RX821002, inhibition of [3 H]-RX821002 and [3 H]-yohimbine by UK14304 was performed to appreciate the relative proportion of high and low affinity state receptors defined by the ligands (Table 3). Data obtained in these experiments show that the percentage of high affinity state receptors was slightly lower with [3 H]-RX821002 than with [3 H]-yohimbine (21 to 34%). Thus, the difference in $B_{\max}$ values may be ascribed to a better labelling of the low affinity state receptor by [3 H]-RX821002. At the time, there is no suitable explanation for the higher labelling of [3 H]-RX821002. However, a similar observation has been reported by our group on the α_2 -adrenoceptor described on the human fat cell (Galitzky *et al.*, 1990) and the adenocarcinoma HT29 cell (Langin *et al.*, 1989). On the human fat cell, guanosine 5'-triphosphate (GTP) addition in the binding buffer increases the $B_{\max}$ values estimated with [3 H]-yohimbine without changing those obtained with [3 H]-RX821002. Nevertheless, GTP was without effect on [3 H]-yohimbine binding on the HT29 cell line α_2 -adrenoceptor ($B_{\max}$ value unchanged). Similarly, GTP did not modify the binding capacities of the two tritiated antagonists on human platelet α_2 -adrenoceptor (not shown). Although questions remain to be answered, these observations indicate that under classical binding conditions, [3 H]-yohimbine probably does not label the whole α_2 -adrenoceptor population.

Results obtained from competition studies at α_2 -adrenoceptors from different tissues have supplied pharmacological evidence for the existence of α_{2A} - and α_{2B} -adrenoceptor subtypes (Bylund, 1985; Bylund *et al.*, 1988). Pharmacological distinction between the two subtypes is essentially based on the relative affinity of oxymetazoline and prazosin for these sites: oxymetazoline is more selective for the α_{2A} -subtype whereas prazosin is relatively selective for the α_{2B} -receptor. Competition studies with [3 H]-RX821002 binding by oxymetazoline, yohimbine and prazosin performed here showed that oxymetazoline and yohimbine have a high affinity for the human platelet α_2 -receptor whereas prazosin

competes poorly with the binding of both radioligands (Figure 4). Thus, [^3H]-RX821002 can label a typical α_2 -adrenoceptor; the order of potency agrees with Bylund's classification and confirms the α_{2A} nature of the human platelet α_2 -adrenoceptor (Bylund, 1985; Bylund *et al.*, 1988).

To conclude, these data demonstrate that a new selective α_2 -adrenoceptor, antagonist, RX821002, exhibits a higher potency than yohimbine in inhibition of adrenaline-induced platelet aggregation. When compared to [^3H]-yohimbine, studies carried out with its tritiated form on the human platelet α_{2A} -adrenoceptor indicated that: (1) [^3H]-RX821002

exhibited a higher affinity for the α_2 -adrenoceptor; (2) the non-specific binding of [^3H]-RX821002 was very low; (3) the total number of sites (B_{max} values) defined with [^3H]-RX821002 was significantly higher than that defined with [^3H]-yohimbine. This difference seems to be due to a specific preferential labelling of the low affinity state receptor. This new α_2 -antagonist represents a useful tool for clinical studies of platelet α_2 -adrenoceptors.

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References

- ALEXANDER, R.W., COOPER, B. & HANDING, R.I. (1978). Characterization of human platelet alpha-adrenergic receptor. Correlation of ^3H -dihydroergotryptine binding with aggregation and adenylate cyclase inhibition. *J. Clin. Invest.*, **61**, 1136–1144.
- BARLOW, R.B. (1983). *Biodata Handling with Microcomputers*. Amsterdam: Elsevier Science Publishers.
- BERLAN, M. & LAFONTAN, M. (1985). Evidence that epinephrine acts preferentially as an antilipolytic agent in abdominal human subcutaneous fat cells: assessment by analysis of beta- and alpha2-adrenoceptors properties. *Eur. J. Clin. Invest.*, **15**, 341–348.
- BOON, N.A., ELLIOTT, J.M., GRAHAME-SMITH, D.G., ST JOHN-GREEN, T. & STUMP, K. (1983). A comparison of alpha2-adrenoceptor binding characteristics of intact human platelets identified by [^3H]-dihydroergocryptine. *J. Auton. Pharmacol.*, **3**, 89–95.
- BYLUND, D.B. (1985). Heterogeneity of alpha2-adrenergic receptors. *Pharmacol. Biochem. Behav.*, **22**, 835–843.
- BYLUND, D.B., RAY-PRENGER, C. & MURPHY, T.J. (1988). Alpha-2A and Alpha-2B adrenergic receptor subtypes: Antagonist binding in tissues and cell lines containing only one subtype. *J. Pharmacol. Exp. Ther.*, **245**, 600–607.
- CHEUNG, Y.O., BARNETT, D.B. & NAHORSKI, S.R. (1982). [^3H]-rauwolscine/[^3H]-yohimbine binding to rat cerebral and human platelet membranes: possible heterogeneity of alpha2-adrenoceptors. *Eur. J. Pharmacol.*, **84**, 79–85.
- CONVENTS, A., DE KEYSER, J., DE BACKER, J.P. & VAUQUELIN, G. (1989). Rauwolscine labels alpha2-adrenoceptors and 5-HT1A receptors in human cerebral cortex. *Eur. J. Pharmacol.*, **159**, 307–310.
- COUPRY, I., PODEVIN, R.A., DAUSSE, J.P. & PARINI, A. (1987). Evidence for imidazoline binding sites in basolateral membranes from rabbit kidney. *Biochem. Biophys. Res. Commun.*, **3**, 1055–1060.
- CRAMPES, F., MARCERON, M., BEAUVILLE, M., RIVIERE, D., GARIGUES, M., BERLAN, M. & LAFONTAN, M. (1989). Platelet alpha2 adrenoceptors and adrenergic adipose tissue responsiveness after moderate hypocaloric diet in obese subjects. *Int. J. Obesity*, **13**, 99–110.
- GALITZKY, J., LARROUY, D., BERLAN, M. & LAFONTAN, M. (1990). New tools for alpha2A-adrenoceptor characterization. Identification on membrane and on intact human fat cells using the new antagonist [^3H]-RX821002. *J. Pharmacol. Exp. Ther.*, **252**, 312–319.
- HAMILTON, C.A. & REID, J.C. (1986). Platelet alpha2-adrenoceptors: a valid model for brain or vascular adrenoceptors? *Br. J. Clin. Pharmacol.*, **22**, 623–626.
- JAKOBS, K.M., SAUR, W. & SCHULTZ, G. (1978). Characterization of alpha- and beta-adrenergic receptors linked to human platelet adenylate cyclase. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **302**, 285–291.
- KERRY, R., SCRUTTON, M.C. & WALLIS, R.B. (1984). Mammalian platelet adrenoceptors. *Br. J. Pharmacol.*, **81**, 91–102.
- LANGIN, D. & LAFONTAN, M. (1989). [^3H]-idazoxan binding to non-alpha2-adrenoceptors in rabbit adipocyte membranes. *Eur. J. Pharmacol.*, **159**, 199–203.
- LANGIN, D., LAFONTAN, M., STILLINGS, M. & PARIS, H. (1989). [^3H]-RX821002: A new tool for identification of α_2A -adrenoceptors. *Eur. J. Pharmacol.*, **167**, 95–104.
- LANZA, F. & CAZENAVE, J.P. (1987). Blood platelet adrenergic receptors. Review article. *Nouv. Rev. F. Hematol.*, **29**, 407–424.
- LENEX, R.H., ELLIS, J., VAN RIPPER, D. & EHRLICH, Y.M. (1985). Alpha2-adrenergic receptor-mediated regulation of adenylate cyclase in the intact human platelet: evidence for a receptor reserve. *Mol. Pharmacol.*, **27**, 1–9.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
- MICHEL, C.M., BRODDE, O.E., SCHENPEL, B., BEHRENDT, J., TCHADA, R., MOTULSKY, H.Y. & INSEL, P.A. (1989). [^3H]-idazoxan and some other alpha2-adrenergic drugs also bind with high affinity to a non-adrenergic site. *Mol. Pharmacol.*, **35**, 324–330.
- MOTULSKY, M.J. & INSEL, P.A. (1982). [^3H]-dihydroergocryptine binding to alpha-adrenergic receptors of human platelets: a reassessment using the selective radioligands [^3H]-prazosin, [^3H]-yohimbine and [^3H]-rauwolscine. *Biochem. Pharmacol.*, **31**, 2591–2597.
- NUTT, D.J. (1987). Platelet alpha2-adrenoceptor binding does not predict brain alpha2-adrenoceptor function. *Br. J. Clin. Pharmacol.*, **24**, 122–123.
- SAULNIER-BLACHE, J.S., CARPENE, C., LANGIN, D. & LAFONTAN, M. (1989). Imidazolinic radioligands for the identification of hamster adipocyte α_2 -adrenoceptors. *Eur. J. Pharmacol.*, **171**, 145–157.
- STEER, M. & ATLAS, O. (1982). Demonstration of human platelet beta-adrenergic receptors using 125I-labeled cyanopindolol and ^{125}I -labeled hydroxybenzylpindolol. *Biochim. Biophys. Acta*, **686**, 240–244.
- STEER, M.L., KHORANA, J. & GALGOCI, B. (1979). Quantification and characterization of human platelet alpha-adrenergic receptors using [^3H]-phenolamine. *Mol. Pharmacol.*, **16**, 719–728.
- STILLINGS, M.R., CHAPLEO, C.B., BUTLER, R.C.M., DAVIS, J.A., ENGLAND, C.D., MYERS, M., MYERS, P.L., TWEEDLE, N., WELLBOARN, A.P., DOXEY, J.C. & SMITH, C.F.C. (1985). α -Adrenoceptor reagent. 3. Synthesis of some 2-substituted 1,4-benzodioxans as selective presynaptic alpha2-adrenoceptor antagonists. *J. Med. Chem.*, **28**, 1054–1062.
- VILLENEUVE, A., BERLAN, M., LAFONTAN, M., CARANOBE, C., BONEU, B., RASCOL, A. & MONTASTRUC, J.L. (1985). Platelet alpha2-adrenoceptors in Parkinson's disease: decrease number in untreated patients and recovery after treatment. *Eur. J. Clin. Invest.*, **15**, 403–407.

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